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Short communication

Alkaline extraction of phenolic compounds from intact sorghum kernels

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a major drought-resistant food crop in Africa and Asia. In Western countries, sorghum is utilised as animal feed but is a viable ingredient alternative in gluten-free foods and biofuels (O’Kennedy *et al.*, 2006). The high level of phytochemicals in sorghum kernels is found in the bran and germ (Dykes & Rooney, 2006). A range of phenolic compounds in sorghum offering antioxidant capacity (AOC) includes phenolic acids, flavonoids, anthocyanins and tannins (Awika & Rooney, 2004). Phenolic compounds have been extracted from cereal grains using various techniques such as aqueous alcohol (Medina, 2011; Cuevas Montilla *et al.*, 2011) and enzymes (Cuevas Montilla *et al.*, 2011). However, these methods require grain kernels to be processed into meal or flour prior to analysis. Decortication, a mechanical method, is often cited as a means to isolate the phytochemical-rich bran from the grain (Awika *et al.*, 2005). Yet, decortication is an abrasive method of bran removal and may be less efficient for grains with a softer pericarp (Mwansaru *et al.*, 1988). Researchers have reported success in using alkali solutions to remove bran and improve milling yields in corn (Blessin *et al.*, 1970; Mistry & Eckhoff, 1992), but in the literature, there are no available data concerning the evaluation of the waste streams obtained by a chemical debranning process for phenolic compounds. The

objective of this study is to explore the application of NaOH extraction for sorghum bran removal and phenolic compounds recovered from the waste stream.

Materials and methods

Chemicals and materials

Sodium hydroxide (NaOH), acetone, sodium phosphate dibasic and sodium phosphate monobasic were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Glacial acetic acid, hydrochloric acid (HCl), Trolox® (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), fluorescein disodium, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), and black opaque and clear 96-well plates were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Four sorghum (three non-tannin and one tannin) samples were chosen based on their colours (white, red, brown and black) and known range of phenolic compound content (low to high). Before treatment, grain hardness for each sorghum sample (fifty kernels) was determined using a single-kernel characterisation system (SKCS 4100) from Perten Instruments, Springfield, IL, USA (Pedersen *et al.*, 1996; Bean *et al.*, 2006). For comparison, other cereal grains (black rice, indigo maize and hard red winter wheat) were included in the analysis to show that AOC could be determined. All the grain samples were supplied by USDA-ARS-Center for Grain & Animal Health Research, Manhattan, KS, USA.

Sodium hydroxide extraction

Phenolic compounds were extracted using a modified method by Lazaro & Favier (2000). Approximately

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Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

5 g of grain was steeped in 20 mL of 10% NaOH while being shaken and incubated (Labnet International Vortemp 1550, Edison, NJ, USA) at 60 °C for 10 min. The NaOH extract (supernatant) was reserved for further analysis, and the grains were washed with hot tap water. Alkaline-treated grains were neutralised by vortexing for 5 min in 10 mL glacial acetic acid. The hot water wash was repeated and the grains left to air dry at ambient conditions. NaOH extracts were either neutralised using 6 M HCl to approximately 70% of the original concentration or untreated and diluted to 70% with deionised (DI) water. An aliquot of the neutralised and untreated samples was freeze dried. Prior to analysis, the freeze-dried extracts were rehydrated back to the initial volume with DI water. Liquid samples were analysed for AOC at days 0, 4, 16 and 32. The freeze-dried treatments were analysed 32 days after extraction. Analysis of the freeze-dried samples was delayed to simulate a reasonable storage scenario. Indigo maize, Karl red wheat and black rice were used as a point of initial reference to compare the sorghum treatments. The sodium hydroxide extraction for the other cereal grains was performed using the same procedure as the sorghum; however, the samples were only analysed on day 0 without carrying out the neutralisation or drying steps.

Sorghum flour extraction

Sorghum flour samples were prepared by grinding the grain using a UDY cyclone mill 181 (UDY Corporation, Fort Collins, CO, USA) with a 0.5-mm screen. The whole-grain flour (0.3 g) was suspended in 10 mL of 75% acetone, shaken for 2 h using a MaxQ 2500 shaker (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20 °C overnight. Extracts were centrifuged at 2900 g for 10 min. The supernatant was saved and the extraction process was repeated the next day on the residues to improve recovery. In the second extraction, 10 mL of 75% acetone and the residues were shaken for 10 min. Both supernatants were pooled for a total volume of 20 mL.

Oxygen radical absorbance capacity

The Oxygen radical absorbance capacity (ORAC) assay was similar to the procedure described by Huang *et al.* (2002). A solution of 153 mM AAPH in 75 mM sodium phosphate buffer (pH 7.4) was prepared fresh daily. A 4×10^3 mM fluorescein stock solution in 75 mM sodium phosphate buffer (pH 7.4) was wrapped in foil and stored at 5 °C. A working solution of diluted fluorescein (1:1000) in 75 mM sodium phosphate buffer (pH 7.4) was prepared daily. Exterior wells of the 96-well plate were filled with 300 µL of DI water. Fluorescein solution (150 µL) was added to all

samples, blanks and standard wells. Solutions (25 µL) of diluted extract, phosphate buffer and diluted Trolox[®] were added to samples, blanks and standard wells, respectively, for a total volume of 175 µL. Equilibration of the plate was performed by incubating at 37 °C for 30 min using a Synergy 2 microplate reader (Biotek Instruments Inc., Winooski, VT, USA). All experimental wells were injected with 35 µL of AAPH solution using the plate reader injector and shaken for 10 s at maximum intensity. Fluorescence was monitored at 485 nm (excitation) and 528 nm (emission) with measurements taken from the top every 60 s for 1 h. ORAC values were calculated as described by Cao & Prior (1999) using the plate reader Gen5 Data Reduction Software (BioTek Instruments) with a standard curve (0–100 µM) of Trolox[®]. ORAC values were expressed in µmol TE per g of grain or flour.

Statistical analysis

All data were plotted and statistical analysis was conducted using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) and OriginPro 8 (OriginLab Corporation, Northampton, MA, USA). Two replications with two subsamples of each treatment were analysed.

Results and discussion

Extraction method

The purpose of this method was to determine the efficacy of an alkaline steeping process for the direct extraction of phenolic compounds from intact whole-grain sorghum. Thus, the benefit of the method is the ability to remove the bran and associated phenolic compounds without a physical debranning process. After the alkaline extraction, no visual colour change was exhibited by the white sorghum, whereas the grain of the red sorghum, Sumac (containing tannin), and black sorghum lost virtually all their colour.

Grain mass

The kernel mass was monitored for 8 days after extraction to assure dryness (data not shown). There was a clear difference in Δ mass between the lighter- and darker-coloured sorghums (Table 1). The white and red sorghum hybrids had little mass loss, whereas the Sumac and black sorghum had a larger mass loss that stayed relatively constant over the week. Differences in Δ mass may be due to variations in pericarp thickness and/or composition between the hybrids (Blakely *et al.*, 1979). Dewar *et al.* (1997) reported that after steeping grain sorghum with dilute NaOH, there was an increase in water uptake, which was seen more in

Table 1 Initial kernel hardness and Δ mass extraction data for four sorghum hybrids

Sorghum name	Pigmented testa layer	Description	Hardness*	Δ Mass % [†]
SP3303	No	White	77.70 \pm 2.53	-4.377 \pm 1.090
MMR	No	Red	76.35 \pm 1.35	-0.167 \pm 0.237
381/73				
Sumac	Yes	Brown, Tannin	65.46 \pm 1.96	-11.337 \pm 0.775
Tx430	No	Black	55.13 \pm 1.80	-15.675 \pm 0.342

*Single-kernel characterisation system (SKCS) hardness index.

[†] Δ Mass % = (mass after drying - mass original)/(mass original) \times 100.

cultivars without tannins. The authors concluded that the NaOH solution reacted with pericarp cell wall material, resulting in higher quantities of phenolic compounds being extracted.

Acetone extraction of flour phenolic compounds

Phenolic compounds from whole-grain sorghum flour were extracted using a 75% acetone solution to show the effectiveness of the NaOH extraction method. An aqueous acetone extraction method was chosen over methanol and ethanol solutions by previous optimisations on sorghum flour (data not shown). Awika *et al.* (2005) employed aqueous acetone for phenol and antioxidant activity on sorghum bran. The AOC was determined on a per gram whole flour basis (Fig. 1). There was no difference between the acetone and NaOH extraction methods for the white and red sorghums. Sumac extracted with NaOH did show a higher ORAC value than the acetone extraction method. Although less evident, the black sorghum (Tx430) also showed similar behaviour. ORAC values were higher in the Sumac treatment possibly because of the presence of tannins that was not found in the

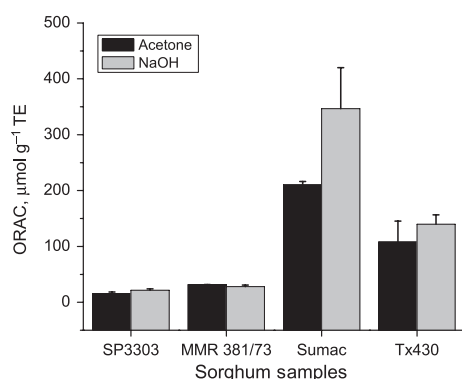


Figure 1 Antioxidant capacity values (day 0) of four sorghum hybrids that were extracted using 75% acetone (whole-grain flour) and 10% sodium hydroxide (intact whole-grain kernels).

other sorghum treatments. Compared to the non-tannin samples, Sumac exhibited the highest AOC in agreement with the trends shown in a review by Awika & Rooney (2004). For AOC analysis, the NaOH extraction was at least equally or more efficient than the acetone method. The data showed that alkaline extraction of phenolic compounds from whole grains may represent a viable alternative to a multistep procedure of decortication and/or milling to flour and subsequently extracted. Folin–Ciocalteu reagent does not react in alkaline conditions; thus, total phenolic content was not reported (Singleton *et al.*, 1999). In addition, the extraction of phenolic compounds from flour could not be effective using the alkaline method because it tends to form clumps, making the results inconsistent.

Antioxidant capacity stability

Stability of the NaOH extracts was determined based on AOC in various conditions (Fig. 2). The liquid extracts were analysed by ORAC at days 0, 4, 16 and 32. All untreated non-tannin sorghums showed more stable AOC values whilst neutralised liquid samples were less stable and lost about half their AOC by day 32 at ambient conditions. Only Sumac showed a decrease in AOC under both conditions. The untreated Sumac extract was the least stable with the original AOC value reduced to about half. Makkar & Becker (1996) reported that the change in chemical structure of the tannin may be due to alkaline treatment. This is critical because tannins are responsible for the AOC (Dykes *et al.*, 2005). Structure change could affect the extractability of the phenolic compounds. If compounds aggregated or formed complexes in the alkali conditions, then it would be more difficult to extract larger compounds; however, if the compounds degrade, there should be no real effect on extractability. There did not appear to be any change in the colour of the extracts over time (no data presented).

Samples of each treated and non-treated NaOH extracts were freeze dried, redissolved to the same volume 32 days after extraction and compared to the original AOC on day 0 (Fig. 3). All the dried samples, either basic or neutral, showed some reduction in AOC. However, the degree of loss in AOC varied for each sorghum hybrid. The dried white and red sorghum samples had little change in AOC. Dried, neutral extracts were the least stable in the Sumac and black sorghums. Further tests would need to be conducted on stability of the NaOH extracts in various conditions such as storage temperature and post-extraction pH.

Sorghum contains various phytochemicals, as referenced earlier, but the majority of focus has been on black and tannin sorghums. A review by Awika &

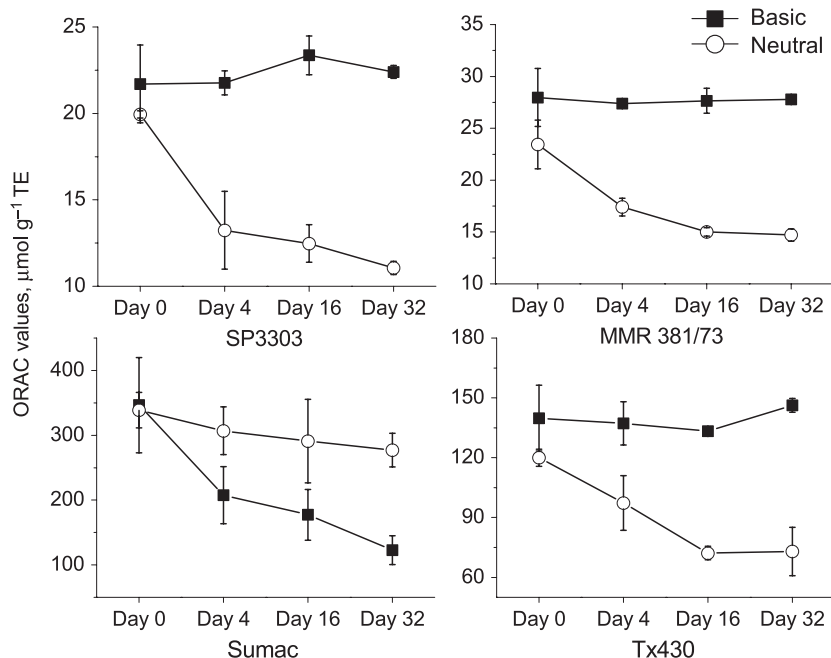


Figure 2 Stability of the oxygen radical absorbance capacity values of neutralised and untreated extracts from sorghum grain during 32 days of storage at ambient temperature.

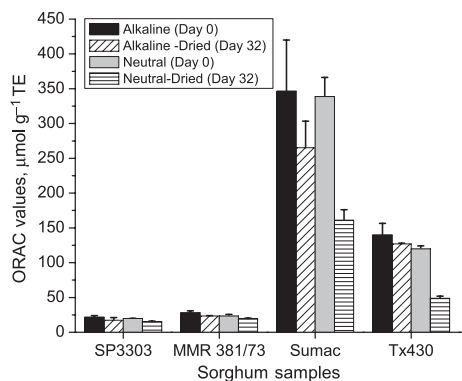


Figure 3 Oxygen radical absorbance capacity values of extracts from sorghum grain that were freeze dried and measured after 32 days.

Rooney (2004) reported higher AOC in black and high tannin sorghum bran than in blueberries and other high antioxidant fruits. Black sorghums have a high amount of anthocyanins particularly 3-deoxyanthocyanidins (Awika & Rooney, 2004). These are different from most anthocyanins because of the lack of a hydroxyl group at the C-3 position with good stability in acidic conditions. Because of the added stability of sorghum anthocyanins, it may be a viable source of anthocyanins for usage as food additives, vitamins or anthocyanin standards.

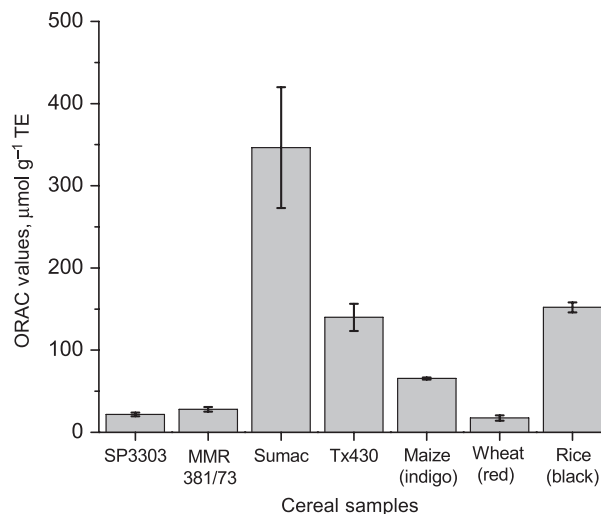


Figure 4 Comparison of oxygen radical absorbance capacity values of sorghum and other selected cereal grains.

Antioxidant capacity comparison amongst grains

To show the efficacy of this extraction method beyond sorghum, the AOC of the four sorghum samples was compared to intact whole-grain indigo maize, red wheat and black rice (Fig. 4). Black rice exhibited the highest AOC value amongst the non-sorghum grains.

Sumac exhibited a twofold higher AOC compared to black rice. Black rice and black sorghum (Tx430) had similar AOC. The data suggests that the NaOH method to extract phenolic compounds from whole grain may be extended to other cereal types.

Conclusions

Direct extraction of the phenolic compounds using an alkaline solvent can be achieved without the need to decorticate or grind the grain. Furthermore, alkaline extraction may hold advantages over the traditional decortications method with grains of soft endosperms. Use of this method could improve the quality whether the goal is pure flour or bran. NaOH would remove colour as well as bran, and colour of flour is a measurement of quality. In the mechanical process, the shape of the grain can affect the ability to evenly decorticate. Alkali debranning eliminates this, lowering the amount of rejected misshapen grain. Improving quality, yield and percent rejected grain can increase revenue. One disadvantage is the interference with Folin–Ciocalteu reagent for the total phenolic assays although it is clear from the ORAC values that phenolic compounds are being extracted. AOC was stable for at least a month, and the method is applicable to other cereal grains. Implementing this method for routine analysis would require further optimisation as far as treatments and the types of information desired.

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